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TECHNICAL MANUSCRIPT 373

EFFECT OF INPUT MULTIPLICITY AND TISSUE CELL CONCENTRATION UPON THE GROWTH OF RIFT VALLEY FEVER VIRUS

Michael D. Orlando
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MARCH 1967

DEPARTMENT OF THE ARMY
Fort Detrick
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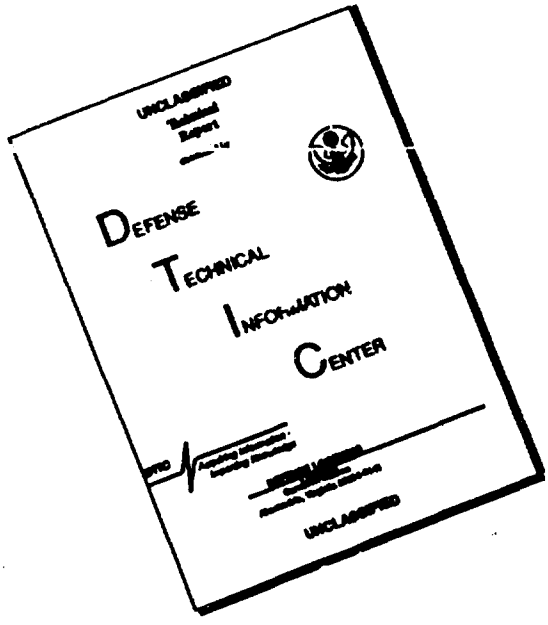

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EFFECT OF INPUT MULTIPLICITY AND TISSUE CELL CONCENTRATION UPON THE GROWTH OF RIFT VALLEY FEVER VIRUS

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Project 1B533601D426

March 1967

ABSTRACT

The growth of Rift Valley fever (RVF) virus in L (Earle) cells obtained from two sources and the effect of input multiplicity and tissue cell concentration upon the growth of the virus are described. The titers obtained in the suspension cultures with the cells from two laboratories were significantly different. With both monolayer culture system and suspension culture system, an input multiplicity of 2.5 resulted in the greatest proliferation of virus. Optimal viral yield was obtained in suspension cultures with 40×10^6 tissue cells per 100 ml of suspension.

I. INTRODUCTION

Although many papers have been presented on Rift Valley fever (RVF) virus since its isolation by Daubney, et al.[1] work on the growth of RVF virus in tissue culture systems has been meager. Easterday and Murphy[2] described the growth of RVF virus in five established cell lines under a limited set of conditions. Coackley[3] described the alteration in virulence during serial passage in lamb testis cells. Tribble and Boyle[4] studied the growth of RVF virus in L (Earle) cells in suspension cultures of 30-ml volume in Nagle's defined medium. However, the effect of input multiplicity (ratio of inoculum to tissue cells) and tissue cell concentration were not reported.

This report describes the growth of RVF virus in L cells obtained from two sources and the effect of input multiplicity and tissue cell concentration upon that growth.

II. MATERIALS AND METHODS

A. VIRUS STRAIN

The pantropic Van Wyk strain of RVF virus described by Kashula[5] was used. Infected lamb serum stored at -70 C was the working seed stock. It had a titer of about $10^{8.3}$ mouse intracerebral median lethal doses ($MICLD_{50}$) per ml.

B. TISSUE CULTURE SYSTEMS

The established L cell line was obtained from two laboratories. In laboratory 1, the cell line had been maintained in suspension cultures[6] on Eagle's minimum essential medium (double strength) with Earle's balanced salt solution supplemented with bovine serum (10%), cysteine (260 µg/ml), and ascorbic acid (50 µg/ml). In laboratory 2, the cell line had been maintained as monolayers with medium 199 (described by Morgan et al.[7]) supplemented with 10% calf serum.

Cultures were maintained in our laboratory as either monolayers or suspension cultures at 36 C. The monolayer cultures were prepared in roller bottles* (650 ml volume) rotated at 7 rpm. The total cell concentration in the roller bottle was 2.8×10^7 cells with 80 ml of growth medium. Suspension cultures were prepared in 100-ml volumes

* Strumia blood plasma bottles obtained from Arthur Thomas Co., Philadelphia, Pa.

in centrifuge bottles (250-ml) fitted with Teflon-coated, suspended bar magnets and removable vents.* Medium 199 supplemented with 10% calf serum was used as the growth medium. Penicillin (250 units per ml), dihydrostreptomycin (250 μg/ml), and kanamycin (50 μg/ml) were added to the growth medium during the viral infectivity studies only.

C. INFECTION OF TISSUE CULTURES

The roller bottles and suspension cultures were infected with sufficient inoculum in a total volume of 10 ml of growth medium to obtain input multiplicities of 1, 2.5, 5, and 10.

The suspension cultures were started with total cell populations of 20, 40, 80, 120, 160, and 200 million cells. The amount of inoculum varied with the total cell population so that a constant input multiplicity of 2.5 was maintained. The culture systems were incubated at 36 C.

D. ASSAY

Tenfold serial dilutions were made in medium 199 plus 10% calf serum. Ten- to 14-gram Swiss-Webster mice were injected intracerebrally with 0.03-ml amounts of appropriate virus dilutions. Ten mice were injected per dilution, and deaths were recorded during the next 6 days. Deaths prior to 24 hours were assumed to be traumatic. The LD_{50} values were calculated by the method of Reed and Muench.[8]

III. RESULTS

The results of the study (means of four replicates) on the effect of input multiplicity are presented in Table 1. In the roller bottle (monolayer system) the effect of input multiplicity can readily be seen by a comparison of the output factor (yield titer divided by inoculum titer) as follows:

Input Multiplicity	Output Factor
1	40
2.5	40
5	8
10	5

* Personal communication, J. Rosensteel and Wm. F. Daniels.

TABLE 1. EFFECT OF INPUT MULTIPLICITY UPON THE GROWTH OF RIFT VALLEY FEVER VIRUS

Input Multiplicity	Titers, $MICLD_{50}$ per ml						
	Roller Bottles[a] at Indicated Time, hr		Suspended Cultures[b] at Indicated Time, hr				
	0	72	0	24	48	72	96
1	6.00	7.60	6.60	[c]	6.44	6.30	5.34
2.5	6.40	7.99	6.90	6.51	6.31	7.17	5.64
5	6.70	7.65	7.20	7.50	6.80	6.74	5.82
10	7.00	7.69	7.50	7.26	6.70	6.73	6.27

a. Results are the means of the titers obtained from cells from both laboratories (no significant difference between cell sources).
b. Results are those obtained with cells from laboratory 2 only.
c. Samples lost.

On the basis of the output factor, the use of either the 1- or 2.5-input multiplicity produces the maximum growth ratio, but on the basis of the largest yield per unit volume ($MICLD_{50}$/ml) the 2.5-input multiplicity is optimal.

The results of the study on input multiplicity in suspension cultures were disappointing. Apparently the only input multiplicity that resulted in a proliferation of the virus occurred with the 2.5 ratio after 72 hours (an increase of only 0.27 log).

The results of the study (means of four replicates) on the effect of tissue cell concentration are summarized in Table 2 on the basis of titers obtained after 72 hours of incubation. There were no significant differences in the titers for each cell source. However, the titers obtained with cells from the two laboratories were significantly different. At each cell concentration, the titers obtained with the cells from laboratory 2 were greater than those obtained with cells from laboratory 1.

The effect of tissue cell concentration can be seen more readily by a comparison of the output factor. The optimal yield was obtained at a total suspension concentration of 40 million cells per 100 ml of suspension with cells obtained from laboratory 2.

TABLE 2. EFFECT OF TISSUE CELL CONCENTRATION OF SUSPENSION CULTURES UPON THE GROWTH OF RIFT VALLEY FEVER VIRUS

Cell Concentration, 10^6 cells per suspension	Titer, $MICLD_{50}$		Output Factor			
			Per Tissue Culture Cell		Per Viral LD_{50} of Inoculum	
	Lab 1	Lab 2	Lab 1	Lab 2	Lab 1	Lab 2
20	6.30	6.81	10	32	4	13
40	6.87	7.45	19	70	8	28
80	6.56	7.16	5	18	2	8
120	6.31	7.42	2	22	1	13
160	6.26	7.16	1	9	>1	4
200	-[a]	7.16	-	7	-	3

a. Samples gelatinous and could not be resuspended in diluent.

IV. DISCUSSION

Although the source of L cells did not affect the growth of RVF virus in the monolayers, a significant effect was seen in the suspension cultures. With either system, the monolayer or the suspension culture, the optimal input multiplicity is apparently 2.5. Although the difference in input multiplicity varied tenfold there was a complete destruction of the tissue cells by 72 hours in the monolayer systems.

The optimal tissue cell concentration in the suspension system is apparently 40×10^6 cells per 100 ml. Many factors may influence proliferation of the virus. Since the suspension system was not designed with precise controls, the reasons for the optimal growth at this tissue cell concentration cannot be specified. If the suspension study had been conducted in a "controlled" device as described by Daniels et al.[6] a better understanding of the metabolic effects of viral infection could have been reached. Since cytolytic viruses alter the biosynthetic processes of the tissue system one could then study the changes that occur in the RVF virus infection of tissue cells.

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14. Key Words

*Rift Valley Fever
*Growth
*Tissue Cultures
L Cells
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